1. INTRODUCTION

Aquaculture is the fastest growing food sector globally and is established itself as high protein resource to fulfil the food demand as the natural resources are facing over exploitation. India ranks third in the global aquaculture production with 4884.0 thousand tonnes and it stands as the second leading producer of finfish with 4391.1 thousand tonnes and fourth in global crustacean production with 385.7 thousand tonnes (FAO statistics., 2014).

Fig 1 Global aquaculture production (FAO, 2014)

Aquaculture in India has evolved as a viable commercial farming practice and it has shown an impressive annual growth at a rate of 6-7 percent. Aquaculture industry in India has been dominated by finfish and shrimp cultures. In case of shrimp aquaculture it has shown a tremendous growth and achieved highest production (4,34,558 MT) in the year 2014-15 (MPEDA., 2014-15). However, the intensification of aquaculture of has led to outbreaks of various diseases, incurring huge economic losses to the aquaculture industry worldwide. There are various diseases associated with aquaculture which has different aetiological
agents like virus, bacteria, fungi and parasites. OIE has listed the most serious pathogens that are prevailing among the aquatic animals. They are listed below.

**Table 1. OIE listed pathogens of Finfish, Crustaceans and Molluscs**

<table>
<thead>
<tr>
<th>Finfish</th>
<th>Crustacean</th>
<th>Mollusc</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Epizootic haematopoietic necrosis disease</em></td>
<td><strong>Acute hepatopancreatic necrosis disease</strong></td>
<td><em>Infection with abalone herpesvirus</em></td>
</tr>
<tr>
<td>**<em>Infection with Aphanomyces invadans (EUS)</em></td>
<td>*<strong>Crayfish plague (Aphanomyces astaci)</strong></td>
<td><em>Infection with Bonamia exitiosa</em></td>
</tr>
<tr>
<td><em>Infection with Gyrodactylus salaris</em></td>
<td><em>Infection with Yellowhead virus</em></td>
<td><em>Infection with Bonamia ostreae</em></td>
</tr>
<tr>
<td><em>Infection with HPR0 infectious salmon anaemiarus</em></td>
<td><em>Infectious hypodermal and Haematopoietic necrosis</em></td>
<td><em>Infection with Marteilia refringens</em></td>
</tr>
<tr>
<td><em>Infection with salmonid alphavirus</em></td>
<td><em>Infectious myonecrosis</em></td>
<td><em>Infection with Perkinsus marinus</em></td>
</tr>
<tr>
<td><em>Infectious haematopoietic necrosis</em></td>
<td><strong>Necrotising hepatopancreatitis</strong></td>
<td><em>Infection with Perkinsus olseni</em></td>
</tr>
<tr>
<td><em>Koi herpesvirus disease</em></td>
<td><em>Taura syndrome</em></td>
<td><strong>Infection with Xenohaliotis californiensis</strong></td>
</tr>
<tr>
<td><em>Red sea bream iridoviral disease</em></td>
<td><em>White spot disease</em></td>
<td></td>
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<tr>
<td><em>Spring viraemia of carp</em></td>
<td><em>White tail disease</em></td>
<td></td>
</tr>
<tr>
<td><em>Viral haemorrhagic septicaemia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epizootic haematopoietic necrosis disease</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* - virus, ** - bacteria-, *** fungi-, 'parasite)

As the bacterial pathogen can survive well in aquatic environment independently of their hosts, bacterial diseases have become a major constrain to aquaculture development especially in case of tropical countries. So far, bacterial species belonging to at least 13 genera have been reported to be pathogenic to aquatic animals. The pathogenic bacteria divided into two groups based on Gram staining characteristics. In case of Gram negative bacteria it includes *Aeromonas, Edwardsiella, Flavobacterium, Francisella, Photobacterium, Piscirickettsia, Pseudomonas, Tenacibaculum, Vibrio* and *Yersinia* and Gram positive bacteria include *Lactococcus, Renibacterium* and *Streptococcus*. Feeding infected fish with antibiotic-medicated food is a general practice of therapeutics for controlling bacterial diseases but it has led to antibiotic resistance development in bacterial pathogen, resulting in a higher dose requirement for effective control which is a matter of increasing public concern. Antibiotics are a group of natural or synthetic compounds that kill bacteria or inhibit their growth. As a result of the unhygienic stressful conditions present in aquaculture facilities like high fish densities, the risk of bacterial infections among the cultured fish is high. Therefore, heavy amounts of antibiotics are administered in fish feed for prophylactic (disease prevention) and therapeutic (disease treatment) purposes. Thus, the antibiotic abuse ultimately leads to several problems. One of such is the development of antibiotic resistant bacteria that arises through mutations in bacterial...
DNA or through horizontal gene transfer mechanisms including conjugation with other bacteria, transduction with bacteriophage, and the uptake of free DNA via transformation.

According to Schmieder and Edwards (2012) there are four well known mechanisms that enable bacteria to withstand antibiotics. They are

1) Inactivation or modification of the antibiotic.
2) Alteration in the target site of the antibiotic that reduces its binding capacity.
3) Modification of metabolic pathways to circumvent the antibiotic effect.
4) Reduced intracellular antibiotic accumulation by decreasing the permeability and/or increasing the active efflux of the antibiotic.

Thus, there are some antibiotics which have been authorized by FAO especially for aquaculture usage. The list is given below (Sapkota et al., 2008).

**Table 2. List of Antibiotics authorized by FAO for Aquaculture**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>• Furunculosis in salmonids caused by <em>Aeromonas salmonicida</em>.</td>
</tr>
<tr>
<td></td>
<td>• Hemorrhagic septicaemia caused by <em>Aeromonas hydrophila, A. sobria</em> and</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em>.</td>
</tr>
<tr>
<td></td>
<td>• Cold water disease in salmonids, caused by <em>Cytophaga psychrophilia</em>.</td>
</tr>
<tr>
<td></td>
<td>• Columnaris disease in salmonids, caused by <em>Flexibacter columnaris</em>.</td>
</tr>
<tr>
<td></td>
<td>• Enteric redmouth disease, caused by <em>Yersinia ruckeri</em></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>Indicated in the treatment of furunculosis caused by susceptible strains of</td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas salmonicida</em>.</td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td>Indicated in the treatment of furunculosis, vibriosis and enteric redmouth in Salmonidae</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>• Bacterial kidney disease (<em>Renibacterium salmoninarum</em>)</td>
</tr>
<tr>
<td></td>
<td>• Streptococcusis in yellowtail.</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>Furunculosis, enteric redmouth disease and vibriosis.</td>
</tr>
</tbody>
</table>

Although only five antibiotics have been approved by FAO for aquaculture use, the abuse of antibiotics is having a high prevalence among the aquaculturist. Antibiotics like chloramphenicol have been banned in many countries but it plays an important part in abuse of antibiotics. The most widely used antibiotic is Oxytetracycline followed by chloramphenicol and oxolinic acid, while sarafloxacin and sulfadimidine was the least used antibiotic (Sapkota et al., 2007). Hence, abuse of antibiotics should be avoided to take a step towards sustainable aquaculture.

Various prophylactic measures have been developed recently to combat diseases in aquaculture. Of these, probiotics which are live microbial feed supplement that beneficially affects the aquaculture systems by
improving both host’s intestinal balance and water quality. Generally, probiotic strains have been isolated from indigenous and exogenous microbiota of aquatic animals. For development of probiotics, the dependence on classical microbiological techniques has hindered for a long time as only a minimal amount of these beneficial microbes can be cultured. It is widely accepted that up to 99.8% of the microbes present in many environments are not readily culturable (Streit et al., 2004). So, this makes a lot of beneficial microbiota unavailable for scientific scrutiny by the biotechnological researches. In recent years, molecular technologies have stepped in to explore the diversity and potential of microbial communities and to overcome the difficulties and limitations associated with culture techniques. Metagenomics is one of the culture independent molecular techniques in which the microbial community can be analysed completely without any biasness.

2.PROBIOTICS

In the recent years considerable attention has been given to the alteration of the gut microbiota to boost health of the host through use of probiotics. A probiotic is defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). In the year 1991, Kozasa made the first empirical application of probiotics in aquaculture. He used spores of *Bacillus toyoi* as feed additive to increase the growth rate of yellow tail, *Seriola quinquerradiata*.

A. Effects of probiotics to the host

A number of specific modes of action by probiotic microorganisms have been attributed to physiological benefits in fish. Although gut colonization is often identified as the most important characteristic of an effective probiotic, the reality is that the benefits incurred by the host from probiotic supplementation are likely a synergistic product of multiple biological effects in which some properties are independent of gut colonization. It includes production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, inhibition of virulence gene expression or disruption of quorum sensing, enhancement of the immune response, source of macro and/or micronutrients, enzymatic contribution to digestion, and stimulation of local and systemic immune responses. The immunomodulation of host by the probiotic strain is important in combating the bacterial pathogens. The innate immune system, also known as non-specific immune system is the first line of defense which comprises the cells and mechanisms that defend the host from infection by other organisms. According to Akthar et al., (2015) probiotics interact with the immune cells such as mononuclear phagocytes (monocytes, macrophages) and polymorphonuclear leukocytes (neutrophils), natural killer (NK) cells, to enhance innate immune responses. Some probiotics can increase the number of erythrocytes, granulocytes, macrophages and lymphocytes in various fishes.
Increasing the level of immunoglobulin by probiotic supplementation has also been observed (Kim et al., 2006).

B. Host immune system and probiotics

Various beneficial effects of probiotic on host’s innate immune system have been documented (Akthar et al., 2015)

a) Phagocytic activity
Phagocytes such as neutrophils and macrophages play an important role in the anti-bacterial defense. These cells ingest and kill bacteria through the production of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and hydroxyl radicals in the respiratory burst. Therefore, probiotics can effectively improve phagocytes in host and improve phagocytosis activity by lactic acid bacteria (LAB) group of probiotics such as *Lactobacillus rhamnosus*, *L. acidophilus* and *L. lactis* which has been reported in several animals.

b) Respiratory burst activity
Respiratory burst activity is important innate defense mechanisms of fish. Probiotics have been reported to improve the respiratory burst of phagocytic cells, which play a central role in the protection of non-specific cell. Lactic acid bacteria significantly improved the respiratory burst of peripheral blood lymphocytes (PBL) in fish. Several *in vitro* and *in vivo* studies showed a significant increase in respiratory burst activity by various probiotics in many aquatic animals and fish.

c) Lysozyme
Lysozyme is a component of the innate immune system and plays an important role in the defense mechanism because of its anti-cancer, anti-viral and opsonization properties. It is one of the most important bactericidal enzymes of intrinsic immunity and is an indispensable tool for the fish to fight infectious agents. Probiotics are found either singly or in combination to trigger the lysozyme level in bony fish. Probiotics like *Lactobacillus rhamnosus*, *Kocuria* sp and *Carnobacterium* sp aid in triggering lysosomal activity.

d) Peroxidase Activity
The peroxidase is an important enzyme which uses oxidizing radicals to kill pathogens. During the oxidative respiratory burst, peroxidase is usually released from the granules of neutrophils. Nutritional supplements of probiotics such as *Bacillus subtilis*, alone or in combination with *Lactobacillus delbrueckii*
spp can induce peroxidase activity. Likewise, probiotics such as Enterococcus faecium also can increase the serum level of peroxidase.

e) Antiprotease
Antiprotease activity in serum, which contains α1-antiprotease, the α2-antiprotease and α2 macroglobulin, inhibits the activity of the proteases used by certain bacteria to invade the host. This activity is generally high and is not affected by immunization or infection. A significantly higher anti-protease activity is observed when probiotic species, Kocuria is supplemented.

f) Complement System
The complement system is the part of the immune system that helps or supplements the ability of phagocytes to combat pathogens by antibody opsonization. Complement, is the main component of the innate humoral immune response, that plays an essential role in the modification of the host immune system to fight against the potential pathogens and their clearance. Complement is initiated by one or a combination of the three pathways, namely, the classical, alternative and lectin pathway. All three paths merge into a common amplification with C3 and navigate through the terminal path that leads to the formation of the membrane attack complex, which can directly lyse pathogenic cells. Serum C3 and C4 levels of complement protein were significantly higher in probiotic treated fish. The probiotic Pediococcus acidilactici when used as a potent food additive improves the level of the alternative complement activity in the serum.

g) Cytokines
Cytokines are a broad and loose matter of small proteins that are important in cellular signaling. They are released by cells and affect the behavior of other cells, and sometimes producing cell itself. Cytokines include chemokines, interferons, interleukins, lymphokines, tumor necrosis factor. A number of probiotics can effectively modulate the production of pro-inflammatory cytokines, such as interleukin-1, tumor necrosis factor α and interferon-gamma and anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (TGF β) in many organisms. Probiotics such as L.rhamnosus, E.faecium and B. subtilis are found to up regulate cytokines.

Figure 2. Expression of cytokines through recognition of microbe associated molecular pattern (MAMP) by pattern recognition receptor (PRR)
Modes of action of probiotics in gut epithelium

Probiotic organisms can provide a beneficial effect on intestinal epithelial cells in numerous ways. It includes

1. It can block pathogen entry into the epithelial cell by providing a physical barrier, referred to as colonization resistance.
2. It create a mucus barrier by causing the release of mucus from goblet cells.
3. Probiotics maintain intestinal permeability by increasing the intercellular integrity of apical tight junctions.
4. Probiotic strains have been shown to produce antimicrobial factors.
5. Probiotics stimulate the innate immune system by signaling dendritic cells producing cytokines.

Figure 3 Different modes of action by a probiotic in the gut epithelium

C. Applications of Probiotics in Aquaculture

The need for sustainable aquaculture has promoted research into the use of probiotics on aquatic organisms. The initial interest was focused on their use as growth promoters and to improve the health of animals. However, new areas have been found, such as their effect on stress tolerance. The major applications of probiotics in aquaculture are

a) Growth Promoter

Probiotics have been used in aquaculture to increase the growth of cultivated species, in reality it is not known whether these products increase the appetite, or if, by their nature, improve digestibility. According to Balcazar et al (2006) probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so as probiotics constantly added to fish cultures, they adhere to the intestinal mucosa,
developing and exercising their multiple benefits. This also depends on factors such as hydrobionts species, body temperature, enzyme levels, genetic resistance, and water quality.

b) Inhibition of Pathogens
Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, thus constituting a barrier against the proliferation of opportunistic pathogens. In general, the antibacterial effect is due to one or more of the following factors: production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases) and/or hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids.

c) Improvement in Nutrient Digestion.
Probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extracellular enzymes such as proteases, amylases, and lipases as well as provide growth factors such as vitamins, fatty acids, and aminoacids. Therefore, nutrients are absorbed more efficiently when the feed is supplemented with probiotics.

d) Improvement of Water Quality
By maintaining high levels of probiotics in production ponds, the accumulation of dissolved and particulate organic carbon can be minimized during the growing season. In addition, this can balance the production of phytoplankton. In several studies, water quality was recorded during the addition of probiotic strains especially of the gram-positive genus Bacillus. Probably since this bacterial group is more efficient than gram-negative in transforming organic matter to CO2 (Cruz et al., 2012). Probiotics has several applications in aquaculture which is tabulated below (Cruz et al., 2012)

Table 3. List of probiotics and their applications on different hosts

<table>
<thead>
<tr>
<th>Application</th>
<th>Probiotic strain</th>
<th>Benefited host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth promoter</td>
<td><em>Bacillus</em> sp.</td>
<td><em>Penaeus monodon</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em> sp.</td>
<td><em>Catfish</em></td>
</tr>
<tr>
<td></td>
<td><em>Carnobacterium divergens</em></td>
<td><em>Gadus morhua</em></td>
</tr>
<tr>
<td></td>
<td><em>Alteromonas</em></td>
<td><em>Crassostrea gigas</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus helveticus</em></td>
<td><em>Scophthalmus maximus</em></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus lactis</em></td>
<td><em>Brachionus plicatilis</em></td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus thermophilus</em></td>
<td><em>Scophthalmus maximus</em></td>
</tr>
<tr>
<td></td>
<td><em>Streptomyces</em></td>
<td><em>Xiphophorus helleri</em></td>
</tr>
<tr>
<td></td>
<td><em>L. casei</em></td>
<td><em>Poeciliopsis gracilis</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em> NL 110, <em>Vibrio</em></td>
<td><em>Macrobrachium rosenbergii</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacillus coagulans</em></td>
<td><em>Cyprinus carpio</em></td>
</tr>
</tbody>
</table>

8
D. Development of probiotics for Aquaculture

Certain properties are required by the probiotic in order to aid in correct establishment of new, effective and safe products. According to Kesarcodi-Watson et al., (2008) the properties include:

1. The probiotic should not be harmful to the host.
2. It should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host.
3. It should reach the location where the effect is required to take place.
4. It should actually work *in vivo* as opposed to *in vitro* findings.

<table>
<thead>
<tr>
<th>Application</th>
<th>Probiotic strain</th>
<th>Benefited host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen inhibition</td>
<td><em>Bacillus</em> sp., <em>Enterococcus faecium</em>, <em>L. rhamnosus</em>, <em>Micrococcus luteus</em>, <em>Pseudomonas fluorescens</em>, <em>P. fluorescens</em>, <em>Pseudomonas</em> sp., <em>Roseobacter</em> sp., <em>Saccharomyces cerevisiae</em>, <em>P. rhodozyma</em>, <em>Vibrio alginolyticus</em>, <em>V. fluvialis</em>, <em>Tetraselmis suecica</em>, <em>Carnobacterium</em> sp., <em>Lactobacillus acidophilus</em>, <em>Bacillus</em> spp., <em>Enterococcus</em> spp., <em>Lactococcus lactis</em></td>
<td>Penaeids, <em>Anguilla Anguilla</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Oncorhynchus mykiss</em>, <em>Scallop larvae</em>, <em>S. exiguis</em>, <em>Litopenaeus vannamei</em>, <em>Salmonids</em>, <em>Oncorhynchus mykiss</em>, <em>Salmo salar</em>, <em>Hepialus gonggaensis larvae</em>, <em>Clarias gariepinus</em>, <em>Farfantepenaeus brasiliensis</em>, <em>Epinephelus coioides</em></td>
</tr>
<tr>
<td>Nutrient digestibility</td>
<td><em>L. helveticus</em>, <em>Bacillus NL 110</em>, <em>Vibrio NE 17</em>, <em>Carnobacterium</em> sp. Hg4-03, <em>Lactobacillus acidophilus</em>, <em>Shewanella putrefaciens</em></td>
<td><em>Scophthalmus maximus</em>, <em>Macrobrachium rosenbergii</em>, <em>Hepialus gonggaensis larvae</em>, <em>Clarias gariepinus</em>, <em>Solea senegalensis</em></td>
</tr>
<tr>
<td>Water quality</td>
<td><em>Bacillus</em> sp., <em>Bacillus NL 110</em>, <em>Vibrio</em> sp., <em>Lactobacillus acidophilus</em>, <em>B. coagulans</em>, <em>Bacillus</em> sp., <em>Saccharomyces</em> sp.</td>
<td><em>Penaeus monodon</em>, <em>Macrobrachium rosenbergii</em>, <em>Clarias gariepinus</em>, <em>Penaeus vannamei</em>, <em>Penaeus monodon</em></td>
</tr>
<tr>
<td>Stress tolerance</td>
<td><em>Alteromonas</em> sp., <em>B. subtilis</em>, <em>L. acidophilus</em>, <em>L. casei</em>, <em>Pediococcus acidilactici</em>, <em>Shewanella putrefaciens</em></td>
<td><em>Sparus auratus</em>, <em>Paralichthys olivaceus</em>, <em>Poecilopsis gracilis</em>, <em>Litopenaeus stylirostris</em>, <em>Makimaki</em></td>
</tr>
</tbody>
</table>
5. It should preferably not contain virulence resistance genes or AB resistance genes.

**Process of designing a probiotic**
The process of designing a probiotic involves five steps. They are

1) Acquisition of bacterial strains,
2) *In-vivo* screening,
3) Pathogenicity test towards target organism,
4) Pilot scale in-vivo screening and
5) Pathogenicity test towards related hosts in the trophic levels. Thus a probiotic is been designed. In case of bacterial strain acquisition, the dependency on classical microbiological methods has biased a large number of unculturable microbes that are found in the environment.

**Figure 4. Steps involved in developing a commercial probiotic strain**

![Diagram of probiotic strain development process]

**3. METAGENOMICS**

Metagenomics is the method in which the genomic analysis can be done to the whole population of microbes. Metagenomics is defined as the direct genetic analysis of genomes contained with an environmental sample. It has become the main technique among the methods which has been designed to get access to the physiology and genetics of uncultured organisms. Direct isolation of genomic DNA from an environment circumvents culturing the organisms under study, and cloning of it into a cultured organism captures it for study and preservation. Advances have derived from sequence-based and functional analysis in samples from water and soil and associated with eukaryotic hosts.
The word metagenomics was first used by Jo Handlesman in 1998 to capture the notion of analysis of a collection of similar but not identical terms such as meta-analysis. Norman Pace was the pioneer in the metagenomics research as he was the first to derive the idea of cloning DNA directly from environmental samples. Then in the year 1991, the first report on cloning in a phage vector directly from environmental samples was reported. The next advance was in the year 1995 with the construction of a metagenomic library with DNA derived from a mixture of prokaryotes enriched on dried grasses in the laboratory. In the following years, Edward DeLong continued the research and defined the field when they reported libraries constructed from prokaryotes in seawater. He identified a 40-kb clone that contained a 16S rRNA gene indicating that the clone was derived from an archaeon that had never been cultured. Following this, much advancement has been established in this technique like the invention of NGS (Next Generation Sequencing) that has dramatically accelerated the development of sequence-based metagenomics. In the future, metagenomics will be used in the same manner as 16S rRNA gene sequencing methods to describe microbial community profiles. It will therefore become a standard tool in the field of microbial ecology.

Thus metagenomics helps to explore the unculturable microbes that has escaped the scientific scrutiny and identify many potential candidates that can be used for probiotic development

A. Technologies involved in Metagenomics

To process a sample in metagenomics the following steps has to be followed. They are

a) DNA extraction from samples.
b) Cloning of the extracted DNA.
c) Sequencing technology in metagenomics
d) Analysis of the metagenome.

a) DNA extraction from samples

In metagenomics, the samples could be processed from any environment, soil or habitat ecosystem. Although various kits were commercially available for DNA isolation from environmental samples, many laboratories have developed their own methods with the aim of optimizing extraction and reducing bias caused by unequal lysis of different members of the soil microbial community. There were two types of extraction techniques. They are

1) Direct, in situ, extraction where the cells are lysed in the soil sample and then the DNA is recovered
2) Indirect extraction techniques, where the cells are removed from the soil and then lysed for DNA recovery.

b) Cloning of the extracted DNA

Following DNA isolation and purification, the cloning of DNA is done using suitable cloning vectors and host strains. The classical approach includes the construction of small insert libraries (<10 kb) in a standard sequencing vector and in *Escherichia coli* as a host strain. However, small insert libraries do not allow detection of large gene clusters or operons. To circumvent this limitation large insert libraries such as cosmid DNA libraries can be employed with insert sizes ranging from 25-35 kb or Bacterial Artificial Chromosome (BAC) libraries with insert up to 200 kb. *E. coli* was still the preferred host for the cloning and expression of any metagenome-derived genes and only very recently have other hosts such as *Streptomyces lividans* been employed to identify genes involved in the biosynthesis of novel antibiotics (Courtois et al., 2003).

c) Sequencing technology in Metagenomics

In the early years commonly followed sequencing protocol in metagenomics is Sanger sequencing. In later years, there was technological advancements with the high throughput sequencing methods such as next generation sequencing methods were used. Two commonly used NGS technologies utilized are the 454 pyrosequencing platform and the Illumina sequencing platform. Here the three commonly used sequencing techniques are discussed. They are

i) Sanger sequencing or chain termination method

ii) 454 pyrosequencing

iii) Illumina sequencing

i) Sanger sequencing or Chain termination method

The key principle of the Sanger method was the use of dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators. The chain termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, radioactively or fluorescently labelled nucleotides and modified nucleotides that terminate DNA strand elongation. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP, dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotide (ddATP, ddGTP, ddCTP, ddTTP) which are the chain terminating nucleotides, lacking a 3’-OH group required for the formation of a phosphodiester...
bond between two nucleotides, thus terminating DNA strand extension and resulting in DNA fragments of varying length. The newly synthesized and labelled DNA fragments are heat denatured, and separated by size by gel electrophoresis with each of the four reactions run in one of the four individual lanes (lanes A, T, G, C), the DNA bands are then visualized by autoradiography or UV light, and the DNA sequence can be directly read off the X-ray film or gel image. A dark band in a lane indicates a DNA fragment that is result of chain termination after incorporation of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP). The relative position of different bands among the four lanes is then used to read (from bottom to top) the DNA sequence.

ii) 454 pyrosequencing platform

The 454 pyrosequencer was the first next-generation sequencer to achieve commercial introduction in 2004. Its chemistry relies on the immobilization of DNA fragments on DNA-capture beads in a water–oil emulsion and then using PCR to amplify the fixed fragments. The beads are placed on a PicoTiterPlate (a fiber-optic chip). DNA polymerase is also packed in the plate, and pyrosequencing is performed. Its main difference from the classic Sanger sequencing is that pyrosequencing relies on the detection of pyrophosphate release on nucleotide incorporation rather than chain termination with dideoxynucleotides. The release of pyrophosphate is conveyed into light using enzyme reactions, which is then converted into actual sequence information.

iii) Illumina sequencing platform

Illumina dye sequencing by synthesis begins with the attachment of DNA molecules to primers on a slide, followed by amplification of that DNA to produce local colonies. This generation of “DNA clusters” is accompanied by the addition of fluorescently labeled, reversible terminator bases (adenine, cytosine, guanine, and thymine) attached with a blocking group. The four bases then compete for binding sites on the template DNA to be sequenced, and the non-incorporated molecules are washed away. After each synthesis cycle, a laser is used to excite the dyes, and a high-resolution scan of the incorporated base is made. A chemical de-blocking step ensures the removal of the 3’ terminal blocking group and the dye in a single step. The process is repeated until the full DNA molecule is sequenced.

d) Analysis of metagenomic library

According to T.J. Sharpton (2014) there are two methods used to analyze the metagenomic data. They are

I. Sequential analysis of metagenomic data

II. Functional analysis of metagenomic data
I. Sequential analysis of metagenomic data

It provides information on the distribution of functions in a community, linkage of traits, genomic organization and horizontal gene transfer. In the case of sequential analysis it is typically quantified by either

1) Analyzing taxonomically informative marker genes,
2) Grouping sequences into defined taxonomic groups (i.e., binning),
3) Assembling sequences into distinct genomes.

These approaches are not mutually exclusive and may be synergistic.

Figure 5. Sequential analysis of a metagenome

1) Marker Gene Analysis

Marker gene analysis is one of the most straightforward and computationally efficient ways of quantifying a metagenome’s taxonomic diversity. This procedure involves comparing metagenomics reads to a database of taxonomically informative gene families (i.e., marker genes), identifying those reads that are marker gene homologs, and using sequence or phylogenetic similarity to the marker gene database sequences to taxonomically annotate each metagenomic homolog. The most frequently used marker genes include rRNA genes or protein coding genes that tend to be single copy and common to microbial genomes. Because this approach involves comparing metagenomic reads to a relatively small database for the purpose of a similarity search (e.g., not all gene families are taxonomically informative), marker gene analysis can be a
relatively rapid way to estimate the diversity of a metagenome. There are two general methods by which marker genes are used to taxonomically annotate metagenomes. The first relies on sequence similarity between the read and the marker genes. The commonly employed bioinformatic tools are MetaPhyler, MetaPhlAn. The second approach uses phylogenetic information, which may take longer to calculate, but may also provide greater accuracy. It then assembles a marker gene phylogeny that includes metagenomic homologs, which are annotated based on their relative location in the tree (i.e., phylotyping). In this method the bioinformatic tools used are AMPHORA, PhylOTU.

2) Binning
A related strategy, known as binning, attempts to assign every metagenomic sequence to a taxonomic group. Generally, each sequence is either classified into a taxonomic group (e.g., OTU, genus, family) through comparison to some referential data or clustered into groups of sequences that represent taxonomic groups based on shared characteristics (e.g., GC content). Binning plays an important role in the analysis of metagenomes. First, depending on the method used, binning may provide insight into the presence of novel genomes that are difficult to otherwise identify. Second, it provides insight into the distinct numbers and types of taxa in the community. Binning algorithms generally come in one of three flavors. They are sequence composition, sequence similarity, and fragment recruitment. Sequence compositional binning uses metagenome sequence characteristics (e.g., tetramer frequency) to cluster or classify sequences into taxonomic groups. These methods generally do not require the alignment of reads to a reference sequence database and, as a result, can process large metagenomes relatively rapidly. Some of these methods instead analyze whole genome sequences ahead of time to train classifiers that stratify sequences into taxonomic groups. PhyloPithia and PhyloPithiaS are the bioinformatic tools used. In case of sequence similarity binning metagenomic reads are binned based on their sequence similarity to a database of taxonomically annotated sequences. Compared to compositional binning tools, these methods tend to require greater computational resources as every read is usually aligned to a large volume of sequences. In addition, these methods, like classification-based compositional binning algorithms, are not necessarily ideal for the identification of novel genomes, though they may be used to identify phylogenetic nodes that contain putatively novel lineages. One of the most widely used bioinformatic tool is MEGAN. In fragment recruitment binning, it identifies reads that exhibit nearly identical alignments to genome sequences (i.e., read mapping) and partitions reads based on the genome to which they map. MOSAIK and Genometa are the commonly used tools.
3) Assembly
Assembly merges collinear metagenomic reads from the same genome into a single contiguous sequence (i.e., contig) and is useful for generating longer sequences, which can simplify bioinformatic analysis relative to unassembled short metagenomics reads. The tools used are meta-IBDA and metavelvet.

II. Function-based analysis of metagenome
Metagenomes provide insight into a community’s physiology by clarifying the collective functions that are encoded in the genomes of the organisms that make up the community. The functional diversity of a community can be quantified by annotating metagenomic sequences with functions. This usually involves identifying metagenomic reads that contain protein coding sequences and comparing the coding sequence to a database of genes, proteins, protein families, or metabolic pathways for which some functional information is known. The function of the coding sequence is inferred based on its similarity to sequences in the database. Doing this for all metagenomics sequences produces a profile that describes the number of distinct types of functions and their relative abundance in the metagenome. In general, metagenome functional analysis involves two non-mutually exclusive steps. They are

1. Gene prediction
2. Gene annotation

1) Gene prediction
Gene prediction determines which metagenomic reads contain coding sequences. Once identified, coding sequences can be functionally annotated. Gene prediction can be conducted on assembled or unassembled metagenomic sequences. For assembled metagenomes with full-length coding sequences, gene prediction is akin to the framework used during the analysis of whole genome sequences with the caveat that some prediction algorithms require species-specific parameters that may not always be appropriate when the contigs have been sampled from diverse or novel lineages. There tend to be three ways by which genes are predicted in metagenomes. They are gene fragment recruitment, protein family classification, and de novo gene prediction.
Fragment recruitment is one of the most straightforward ways of identifying coding sequences in a metagenome. It is used to map metagenomic reads or contigs to a database of gene sequences. Metagenomic sequences that are identical or nearly identical to a full-length gene sequence are considered representative subsequences of the gene. In the case that the gene has a functional annotation, this method of gene prediction can also simultaneously provide a functional annotation for the recruited metagenomic sequences. A related approach involves translating each metagenomic read into all six possible protein coding frames and comparing each of the resulting peptides to a database of protein sequences by sequence alignment. The alignments can then be analyzed to identify those metagenomic sequences that encode translated peptides that exhibit homology to proteins in the database. Bioinformatic tools used are USEARCH and RAPsearch.
**De novo** gene prediction can potentially identify novel genes. Here, gene prediction models, which are trained by evaluating various properties of microbial genes (e.g., length, codon usage, GC bias), are used to assess whether a metagenomic read or contig contains a gene and does not rely on sequence similarity to a reference database to do so. As a result, these methods can identify genes in the metagenome that share common properties with other microbial genes but that may be highly diverged from any gene that has been discovered to date. There are several tools that can be used for **de novo** gene prediction, including MetaGene, Glimmer-MG, MetaGeneMark, FragGeneScan, Orphelia and MetaGun.

2) Gene annotation
Once coding sequences in a metagenome are predicted, they can be subject to functional annotation. The most common way this is accomplished is by classifying the predicted metagenomic proteins into protein families. A protein family is a group of evolutionarily related protein sequences, or subsequences in the case of protein domain families. They are usually characterized by comparing full-length protein sequences that have been identified through genome sequencing projects. Because the proteins in a family share a common ancestor, they are thought to encode similar biological functions. If a metagenomics sequence is determined to be a homolog of this family (i.e., it is classified as being a member of the family), then it is inferred that the sequence encodes the family’s function. Classification of an assembled or unassembled metagenomic protein sequence into a protein family usually requires comparing the metagenomic protein to either a database of protein sequences, each of which is designated as being a member of a family, or comparison of the sequence to a probabilistic model that describes the diversity of proteins in the family. Once the metagenomic sequence has been compared to all proteins or all models, it can either be classified into

- A single family. (e.g., the family with the best hit)
- A series of families. (e.g., all families that exhibit a significant classification score)
- No family, which suggests that the protein may be novel, highly diverged, or spurious.

**Figure 6. Functional analysis of a Metagenome**
4. METAGENOMICS IN THE PROBIOTIC DEVELOPMENT

Until the last decade, our knowledge on microbiota composition and development was largely based on the use of traditional culture-based methods. The use of culturing has provided interesting data but unfortunately also a very biased view of the microbiota composition. The recent development of molecular culture-independent techniques has demonstrated that only a minority of the members of the microbiota are culturable, and therefore most of the microbes present in the microbiome had escaped scientific scrutiny until recently. During the last 20 years, several qualitative and quantitative culture-independent techniques, mostly based on the PCR and DNA hybridization, have been developed and applied to microbiota assessment. More recently, the development of fast and low cost DNA sequencing methods has allowed these modern techniques to be applied to the study of microbial communities directly in their environment without the need for species cultivation and isolation to characterize the microbiome. These techniques, based on extensive sequencing of DNA, have increased enormously our knowledge on microbiome composition and activity. The study of entire microbial communities using metagenomic approaches has revealed a much greater diversity than was previously thought to exist and has helped to determine the community structure of several previously unknown ecosystems. The development of these techniques is also contributing enormously to many different aspects of probiotics research.

According to Guiemondo (2012) metagenomics can aid in probiotic research through

- Defining a healthy microbiome in the host and also identifying aberrancies related to disease and this ultimately leads to new probiotic targets.
- Identifying specific functions related to niche or disease which aids in probiotic selection criteria.
- Finding new microbes and microbial properties that help in identifying new probiotic organism.
- Deciphering the gene expression response to microbiome-microbiome and host-microbiome interaction leading to elucidation of mechanism of probiotics and interaction of probiotics with host.
- Assessing probiotic modulation of microbiome and finding the effects of probiotics leading to assessing the efficacy of probiotic.
- Technological development leading to new methods of probiotic research.

5. MAJOR DISCOVERIES RELATED TO FISH MICROBIOME

Major research has been conducted to study the microbiome of fishes using metagenomics and it has led to many notable findings which involves in both identifying the microbial diversity and functional profiling. A research conducted by Liu et al., (2015) has revealed the diversity of microbiome in wild freshwater fishes in different trophic levels. They have also elucidated the enzyme activities of fishes. The major findings include that
Cellulose-degrading bacteria Clostridium, Citrobacter and Leptotrichiawere dominant in the herbivorous, while Cetobacterium and protease-producing bacteria Halomonas were dominant in the carnivorous. The most abundant phylum was Proteobacteria in all samples, accounting for 45.52% (in herbivorous fish species), 32.82% (in carnivorous fish species), 37.32% (in omnivorous fish species), 38.13% (in filter-feeders) of the total bacterial sequences, indicating that most quantity of gut bacterial species are from this taxon.

Within the Proteobacteria, each fish microbiota was composed of mostly Gammaproteobacteria, followed by Betaproteobacteria and Alphaproteobacteria. Firmicutes was the second most common phylum, accounting for 22.38%, 21.83%, 27.13%, 21.16% in herbivorous, carnivorous, omnivorous, and filter-feeding species.

The gut microbiota of carnivorous fishes possessed a significantly greater abundance of the phylum Fusobacteria (21.91%) compared with filter-feeding fishes (9.41%). Filter-feeding fishes possessed a significantly greater abundance of the phylum Acidobacteria (5.21%) in their gut microbiota compared with carnivorous species (1.08%). Other common taxa were Bacteroidetes, Actinobacteria, Verrucomicrobia, Cyanobacteria, Planctomycetes, Acidobacteria, Crenarchaeota ranging between 0.89% and 8.26% in all the experimental species. Cyanobacteria showed highest abundance in the filter-feeding group compared with other three groups.

Research conducted by Xing et al. (2013) on the gut microbiota of farmed turbot (Scophthalmus maximus) showed that Proteobacteria and Firmicutes were dominant in the turbot GI microbiome. Quorum sensing and biofilm formation was abundant in the turbot GI metagenome. Genes associated with quorum sensing like HapR and Lux genes were found in species within Vibrion, including Vibrion vulnificus, Vibrio cholerae and Vibrio parahaemolyticus. In farmed fish gut metagenomes, the stress response and protein folding subsystems were over represented and several genes concerning antibiotic and heavy metal resistance were also detected. Of these multidrug resistance efflux pumps, fluoroquinolone resistance and cobalt zinc-cadmium resistance were the three largest categories of genes detected.

Figure 7 Microbial diversity among the freshwater fishes at different trophic levels
The study on shrimp microbiome using high throughput sequencing was first done by Rungrassamee et al. (2014) where they characterized the gut microbiome of wild and cultured *P. monodon*. Among them, Proteobacteria (70%) dominated in all shrimp intestines followed by Firmicutes. They also found out that five phyla, *Actinobacteria*, *Fusobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were found in all shrimp from both wild and domesticated environments.

Oetama et al. (2016) analyzed the gut microbiome of *P. monodon* from both cultured and wild shrimps and also detected the pathogenic bacteria associated with *P. monodon*. The bacterial communities belonged to four bacterial phyla which predominantly includes *Proteobacteria* (96.08%), *Bacteroidetes* (2.32%), *Fusobacteria* (0.96%), and *Firmicutes* (0.53%). On the order level profiling *Vibrionales* (66.20%) and *Pseudoaltermonadales* (24.81%) were detected as predominant taxa. qPCR profiling of the order *Vibrionales* was done. It was observed that *V. alginolyticus* and *Photobacterium damselae* were the predominant pathogens present in the shrimp.

Many works on finfish microbiome were performed and it has provided valuable information about the gut microbiota aiding in digestion and stress response. These discoveries could very well lead to identification of potential probiotic strain that could benefit the host in both metabolism and immunomodulation. The findings are listed below (Llewellyn et al., 2014)

**Table 4. Major discoveries in fish microbiome using metagenomics**

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Fish origin</th>
<th>Organ</th>
<th>Major Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Panaque</em> sp. (catfish)</td>
<td>Aquaculture</td>
<td>Faeces samples</td>
<td>Putative cellulolytic bacteria identified: <em>Aeromonas</em> sp., <em>Flavobacterium</em> sp., <em>Bacteroides</em> sp., <em>Clostridium</em> sp., and <em>Pseudomonas</em> sp.</td>
</tr>
<tr>
<td><em>Dorosoma cepedianum</em></td>
<td>Wild</td>
<td>Intestinal mucosa And contents</td>
<td>Significant differences between foregut and hindgut microbiota, but not between species</td>
</tr>
<tr>
<td><em>Hypophthalmichthys molitrix</em></td>
<td>Wild</td>
<td>Intestinal mucosa And contents</td>
<td>Significant differences between foregut and hindgut microbiota, but not between species</td>
</tr>
<tr>
<td><em>Acipenser baerii</em></td>
<td>Aquaculture</td>
<td>Hindgut contents</td>
<td>Arabinofuranosaccharide prebiotics modulate hindgut microbiome composition</td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td>Aquaculture</td>
<td>Hindgut</td>
<td>Microflora enhance fatty acid uptake in the zebrafish intestine</td>
</tr>
<tr>
<td><em>Ctenopharyngodon idella</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa</td>
<td>Cellulose digesting genera present—<em>Anoxybacillus</em>, <em>Leuconostoc</em>, <em>Clostridium</em>, <em>Actinomyces</em>, <em>Citrobacter</em></td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa And contents</td>
<td>Core microbiome: γ-Proteobacteria, β-Proteobacteria, <em>Fusobacteria</em>, <em>Bacilli,</em></td>
</tr>
<tr>
<td>Species</td>
<td>Environment</td>
<td>Sample Type</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Solea senegalensis</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa and contents</td>
<td>Temperature and diet both influence microbiota present</td>
</tr>
<tr>
<td><em>Pelteobagrus fulvidraco</em></td>
<td>Aquaculture</td>
<td>Midgut contents, midgut mucus</td>
<td>Different bacterial genera between gut contents and mucosa. Stomach contents contained Chloroflexi, while mucous Actinobacteria</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em></td>
<td>Aquaculture</td>
<td>Intestinal content</td>
<td>Dietary β-(1,3)(1,6)-D-glucan supplementation impacts gut microbiota</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa and contents</td>
<td>Differences between intestinal mucosa and contents. e.g., Gut contents—Enterobacter, Bacteroides, Flavobacteria, Pasteurellaceae. Mucosa - Enterobacter, Aeromonadaceae, Pseudomonadaceae, Mycoplasmataceae</td>
</tr>
<tr>
<td><em>Dario rerio</em></td>
<td>Aquaculture</td>
<td>Hindgut</td>
<td>Nanoparticles included in diet disrupt community structure</td>
</tr>
<tr>
<td><em>Acipenser baerii</em></td>
<td>Aquaculture</td>
<td>Hindgut</td>
<td>Comparison of different diets on hind gut fermentation</td>
</tr>
<tr>
<td><em>Carassius auratus</em></td>
<td>Aquaculture</td>
<td>Intestinal contents</td>
<td>Antibiotic treatment disrupts microbiota of healthy fish more significantly than those with disease.</td>
</tr>
<tr>
<td><em>Salmo salar</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa and contents</td>
<td>Pseudomonas, Acinetobacter, Flavobacterium, Psychrobacter, Brevundimonas, Caulobacter, Mycoplana, Aeromonas, Haemophilus, Aeromonas salmonicida, Bacillus, Micrococcus/Kocuria. Reduction in diversity among tetracyclin treated individuals</td>
</tr>
<tr>
<td><em>Kyphosus sydneyanus</em></td>
<td>Wild</td>
<td>Intestinal contents</td>
<td>Putative involvement in short chain fatty acid metabolism</td>
</tr>
<tr>
<td><em>Salmo salar</em></td>
<td>Aquaculture</td>
<td>Intestinal content</td>
<td>Addition of soyabean derived protein resulted in dysbiotic changes in intestinal microbiota and presence of genera not normally associated with the marine environment</td>
</tr>
<tr>
<td><em>Gadus morhua</em></td>
<td>Aquaculture</td>
<td>Intestinal mucosa and contents</td>
<td>Dietary differences in microbiota. Bacteriodetes preferentially adherent. Anthrobacter absent from foregut</td>
</tr>
<tr>
<td><em>Salvelinus fontinalis</em></td>
<td>Aquaculture</td>
<td>Skin mucosa</td>
<td>Probiotic treatment by an indigenous strain does not disturb the natural microbiota of <em>Salvelinus fontinalis</em></td>
</tr>
</tbody>
</table>
6. MAJOR POTENTIAL APPLICATIONS OF METAGENOMICS IN AQUACULTURE

Microbial studies in aquaculture are focused on the understanding of the symbiotic and antagonist interrelationships of microbes with eukaryotes such as fish, crustacean and molluscs. In this regard, metagenomics can provide a deeper insight into these relationships by performing associations of the information revealed by the extracted DNA with particular host organisms or ecosystems. Thus Martinez-Porches and Vargas-Albores (2015) has reviewed the applications of metagenomics in aquaculture. They are

a) Identifying novel antibiotic resistant gene
b) Detection of novel viral pathogens
c) Studying microbial communities that are associated with biofloc.

a) Antibiotic resistance genes
The use of antibiotics in aquaculture was perhaps the most popular therapeutic strategy for the treatment of infectious diseases caused by bacteria but its success was compromised by the flowering of tolerance or resistance to a particular compound from the time of its first application. Conventional methods for the detection of antibiotic resistance usually include using growth inhibition assays in broth or agar disc diffusion, in which the minimal inhibitory concentration (MIC) of particular antibiotics could be estimated for each bacterial isolate. One of the limitations of this procedure lies in the fact that only a few bacterial isolates could be studied at a time. The outbreak of technologies such as next-generation sequencing in the scientific community circumvents the above limitations because it is a culture-/amplification independent technique. Therefore, these technologies allow for a deeper insight into the genomic information of most bacteria, leading to the detection of novel resistance genes. The metagenomic approach provides information regarding the presence, absence or modification of the genes responsible for antibiotic resistance. Furthermore, the discovery of novel genes is performed at a faster rate.

b) Detection of viral pathogens
The identification of viral mechanisms may contribute to the rapid identification of pathogenic species and mutations that represent useful information for diagnosis, prevention and the development of treatments. Unfortunately, the identification of pathogens as well as the existing diagnostic methods was circumscribed by an incomplete picture of the enormous viral diversity and the limitations of traditional detection methods. Traditionally, viral pathogens were detected in monolayer cultures that may exhibit cytopathic effects or through antibody neutralization assays. However, antibody neutralization approaches depend upon the availability of antiserum, and most of the viral species were not readily culturable under laboratory conditions which increase the difficulty of identification and discovery of new pathogens as well as their basic study. During the last decades, molecular-based methods such as PCR have been implemented for the study and detection of un-culturable and/or non-isolated viruses. However, PCR-based methods require
previous genomic information, which usually precludes the detection and study of emerging viruses. Shotgun metagenomics of clinical or random environmental samples represents a promising alternative that circumvents the limitations of the traditional methods. Although this technique has been typically used to study genomic diversity, it could also be useful for the identification of viral pathogens in clinical and environmental samples. Studies of viruses using metagenomic approaches have been recently encouraged because of the quality and quantity of genomic information obtained with next-generation sequencing. Viral metagenomics exceeds the coverage and efficiency of any other method used for random identification; moreover, as stated above, metagenomics does not require previous genomic information about any particular pathogen.

c) Microbial communities in relation with formation of bioflocs

The use of microbial biofilms and bioflocs as direct food sources has been exhibited a vigorous growth accompanied by successful results over the last decade. BFT takes advantage of the accumulation of organic residues and the limited water exchange. Thus, BFT has arisen as a successful strategy to take aquaculture intensification and sustainability to the next level. Recent evidence suggests that bioflocs seem to prevent disease outbreaks. As an emergent technology, there is still much to be understood about the biology of bioflocs. In this regard, metagenomics should be used as a tool to detect whether the microbes composing the bioflocs possess gene families encoding for proteins associated with the antagonist activities or whether other mechanisms halt the normal activity of the pathogens.

Conclusion

It has been known for a long time that alterations in gut microbiota of the host are related to certain diseases. However, until recently the beneficial effects of the microbiota on health have been less widely known. These benefits associated with an appropriate microbiota composition and activity constitutes the ‘proof of concept’ for probiotics. Beneficial intestinal microbes constitute the main source for isolation of potential probiotic strains targeted at modulating the host microbiome to restore health. The normal healthy microbiota cannot be easily defined but the development of metagenomics is rapidly extending our understanding of the role the microbiota plays in our health. This is allowing new potential areas of application of probiotics to be identified as well as development of an understanding its mechanisms of action. Combination of genome, microbiome and host-response data promises to bring probiotic research to a new level, in which scientific criteria for selection of effective strains for different conditions will be available worldwide for the scientific community and it helps the aquaculture industry a step ahead towards sustainability.
7. REFERENCES


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